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Desensitization of Central Cholinergic Mechanisms and Neuroadaptation to Nicotine

Enrique L. M. Ochoa,*[*,1] Lan Li,[2] and Mark G. McNamee[2]

[1]Division of Neonatology, Department of Pediatrics, School of Medicine; and [2]Department of Biochemistry and Biophysics, University of California at Davis, Davis, CA 95616

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*Author to whom all correspondence and repnnt requests should be addressed.

Abstract

This review focuses on neuroadaptation to nicotine. The first part of the paper delineates some possible general mechanisms subserving neuroadaptation to commonly abused drugs. The postulated role of the mesocorticolimbic neuroanatomical pathway and drug-receptor desensitization mechanisms in the establishment of tolerance to, dependence on, and withdrawal from psychoactive drugs are discussed.

The second part of the review deals with the pharmacological effects of nicotine at both pre- and postsynaptic locations within the central nervous system, and the still-perplexing upregulation of brain nicotine-binding sites seen after chronic nicotine administration. A special emphasis has been put on desensitization of presynaptic cholinergic mechanisms, and postsynaptic neuronal nicotinic-receptor function and its modulation by endogenous substances. A comparison with the inactivation process occuring at peripheral nicotinic receptors is also included.

Finally, a hypothesis on the possible connections between desensitization of central cholinergic mechanisms and neuroadaptation to nicotine is advanced. A brief comment on the necessity of fully understanding the effects of nicotine on the developing nervous system closes this work.

Index Entries: Neuronal nicotinic receptors; desensitization of pre- and postsynaptic cholinergic transmission; nicotinic receptor upregulation; dependence on nicotine; neuroadaptation to nicotine.

Introduction

Human beings display behaviors that others may consider abusive: They watch too much television, jog excessively, or overeat. They behave that way because of the reward obtained from performing such activities. Other people, instead, use and abuse drugs, and obtain an even more intense reward. Drugs that act on the central nervous system (CNS) have been used for recreational purposes under various cultures and circumstances. Natives all over the Americas ritualistically used centrally active drugs, Sigmund Freud abused tobacco (Cohen, 1988) and indulged in cocaine, and a brand of carbonated soft drink known worldwide contained cocaine as part of its formula at the beginning of this century. It seems clear that a "war against drugs" was not such a compelling enterprise as it seems nowadays.

The reader may legitimately ask: Are those cases typical examples of drug abuse? How does an organized society define drug abuse? What is the difference between abusing a drug and becoming dependent on it? What is the meaning of the words *dependence* and *addiction*, especially within the context of illegal drug consumption? The first part of this review will define these questions. The rest of the paper will be devoted to analyzing the effects of prolonged exposure to nicotine on brain function, and to discussing some testable hypotheses on possible operative mechanisms.

Nicotine was chosen to illustrate some of the basic mechanisms by which addictive drugs might operate on the brain, reflecting the bias of the authors. The laboratory of one of us (M. G. M.) has been devoted to characterizing fundamental principles of nicotinic-receptor function, such as binding of specific ligands, the transduction of this binding into a physiological response, and the agonist-induced inactivation of function, using biophysical, biochemical, and molecular biological approaches.

The main target of nicotine in the brain (i.e., central cholinergic mechanisms) is receiving considerable attention from neuroscientists, mainly as a result of the rapid development that has taken place in the fields of molecular genetics and electrophysiology. At the same time, many of the effects of commonly abused drugs are known to be exerted through specific brain target areas subserved by specific anatomic connections.

The sites to which nicotine binds can now be defined as true nicotinic acetylcholine receptors (nAChRs), members of a family that includes the mammalian skeletal muscle and the electric-fish electroplax receptor. The information on the neurobiology of these receptors spans several levels of organization, ranging from single-channel events to sequence analysis of cloned genes, and many of the cellular processes to which they are associated are well known. In consequence, the study of neuroadaptation to nicotine may prove paradigmatic when analyzing the effects of other centrally acting drugs at the molecular level.

Pre- and postsynaptic cholinergic mechanisms within the CNS are affected by prolonged exposure to nicotine, the major active component of tobacco (Fig. 1). The drug produces behavioral symptoms that range from tolerance, dependence, and withdrawal in the adult (Tennant et al., 1983; Benowitz et al., 1989) to probable attention-deficit disorders and other cognitive syndromes of childhood (Brown et al., 1985; Kristjansson et al., 1989).

Nicotine, in the form of tobacco smoking or chewing, is now included along with other substances, such as alcohol, the opiates, amphetamines, or cocaine, in the category of "addictive" or dependency-producing drugs (Davis, 1987; APA, 1987; DHHS, 1988; Benowitz, 1988; Henningfield and Nemeth-Coslett, 1988; Cohen, 1988; Benowitz et al., 1989). The many other medical conditions associated with the repeated use of nicotine will not be considered here (*see* Benowitz, 1988).

The main focus of this paper is on the hypothetical desensitization of cholinergic mechanisms brought about by a chronic regime of nicotine self-administration, since there is evidence that repeated administration of the drug is associated with inactivation of postsynaptic cholinergic function. It is postulated that long-term changes in this fundamental property of cholinergic neurotransmission produce profound changes in synaptic efficacy, which are ultimately translated into modified behaviors.

This review purports to honor the memory of the late Eduardo De Robertis, a scientist whose achievements contributed to our present understanding of how a chemical synapse operates. De Robertis fostered the birth of neurosciences in his native Argentina, created and directed the Institute of Cell Biology at the Faculty of Buenos Aires, and stimulated the interests of many in brain research, through teaching as well as personal example. In the last years of his life, De Robertis became interested in the effects of psychoactive drugs, especially the benzodiazepines, and his work still continues as a result of the constant efforts made by his group and the endeavors of his many disciples. One of them (E. L. M. O.) feels that this review is timely, helping to keep the memory of "el maestro" alive.

Some Important Definitions

Drugs* that modify behavior are linked to terms like "abuse" and "addiction." It is generally believed that persons abusing psychoactive drugs become *addicted* because they *depend* on the rewarding effects of drugs, which are created by their sustained consumption. Later, these indivi-duals develop *tolerance,* so that they need to increase drug intake in order to get the same subjective degree of satisfaction as was obtained before with lower doses.

Words like "dependence," "tolerance," "addiction," and even "abuse" need to acquire a neurobiological meaning in order to be usefully employed in the present work. For example, patients with congestive heart failure surely *depend* on digitalis, and some people develop *tol-*

*The term drug is used throughout this paper for any agent that, when introduced into a living organism, is able to modify one or several of its functions. The neurotransmitter acetylcholine, the synthetic psychostimulant phencyclidine, the plant alkaloid cocaine, and even water are all examples of drugs. A drug may be also defined as any substance that once injected into an animal produces a scientific paper (attributed to Otto Loewi).

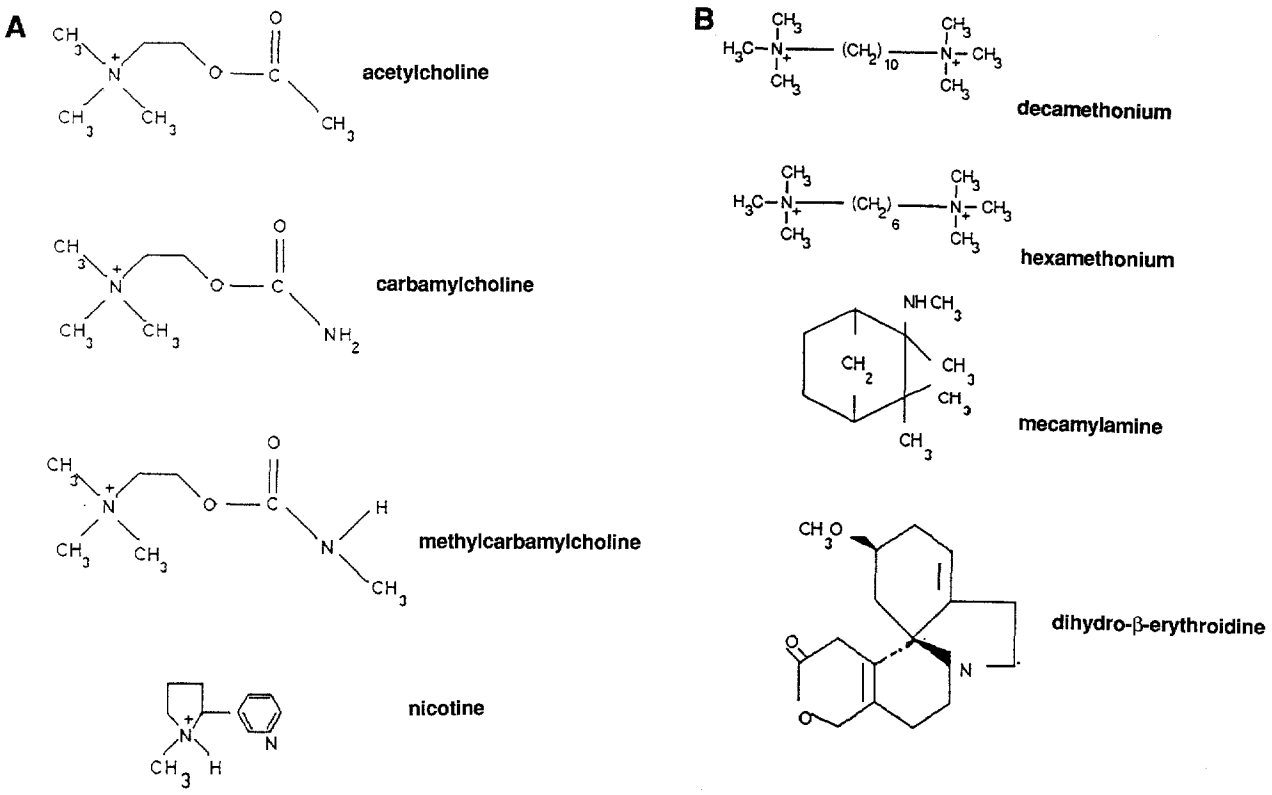

Fig. 1. A: The structures of nicotine; the naturally occurring neurotransmitter acetylcholine; the acetylcholinesterase-resistant carbamylcholine (or carbachol); and the novel agonist methylcarbamylcholine, a selective marker of neuronal nicotinic receptors (Araujo et al., 1988, 1989). B: Three important antagonists of the neuronal acetylcholine receptor: decamethonium, mecamylamine, and dihydro-b-erythroidine. An increase in chain length of only four carbon atoms gives the neuromuscular blocking agent decamethonium. In the past, these ligands were used to discriminate ganglionic (C-6) from end-plate (C-10) receptors. Hexamethonium does not block neuronal nAchRs.

erance to the therapeutic effects of certain antibiotics after prolonged treatments. Are these people really drug addicts? Attempting to describe addiction in terms of dependence on psychoactive drugs implies a need to establish a clear-cut difference between physical and psychic dependence, which in the opinion of many falls within the realm of the heavily disputed (and still unresolved) mind–body problem. Also, the word *abuse* may be misleading because of the social connotations of what is considered abusive, which fluctuates as a function of time, as mentioned above.

In the present work, centrally acting (i.e., psychoactive) drugs will be considered detrimental for a person if their intake is associated with a behavioral triad consisting of tolerance, physical dependence, and withdrawal or the abstinence syndrome. The term *neuroadaptation,* which may or may not encompass this triad, will be defined later.

Tolerance

A person is said to be tolerant to the effects of a psychoactive drug if she or he obtains a diminished response after being exposed to the drug for a defined period of time. Higher intake is required in order to achieve the same effect as that obtained at the initial stages of the abuse period.

Some drugs exhibit the phenomenon of *acute* tolerance, which occurs after a single or a few doses. Smokers develop rapid (minute time range) tolerance to certain effects of nicotine, and this phenomenon is somewhat reversible (Benowitz et al., 1989). However, drugs of abuse (particularly nicotine), are chronically administered so that *chronic* tolerance occurs. Note that tolerance can conceptually be equated with the term *desensitization (see below)*, applied mainly to molecules, cells, and entire organs. Tolerance will be used as a term with behavioral connotations.

It is important to note that not every psychoactive drug produces tolerance, and that drugs that produce tolerance may or may not produce physical dependence. Furthermore, tolerance to one particular effect of a drug may develop in an individual, whereas some other effects of the same drug may show no tolerance at all. Subjects are said to become *cross-tolerant* to several compounds when each compound induces tolerance with respect to the others (e.g., ethanol and nicotine; Collins et al., 1988b; Burch et al., 1988).

Physical Dependence and the Abstinence Syndrome

A person *depends* on a drug if that person feels that she or he really needs the drug in order to continue functioning normally in civilized life. An efficient way to demonstrate that a particular drug makes an individual dependent is to abruptly terminate the administration of the drug and provoke the abstinence syndrome or the withdrawal illness, a complex array of signs and symptoms that generally are the opposite of those originally elicited by the drug (e.g., opiate withdrawal will produce unwanted restlessness rather than the sought-after drug-induced sedation). The abstinence syndrome can be ameliorated by readministration of the discontinued drug.

It is important to note that physical dependence necessarily implies tolerance, but the inverse is not always true (e.g., to be tolerant to an antibiotic does not mean to depend on it, but dependence on opiates necessarily implies tolerance to those drugs). Two or more drugs produce *cross-dependence* when each drug relieves the abstinence syndrome arising from withdrawal from the other.

Neuroadaptation

The triad of tolerance, physical dependence, and withdrawal suggests that a profound change within the brain of the user has occurred after chronic administration of the psychoactive drug, as is the case with the opiates. Other drugs, however, may not show such a full-fledged display of signs and symptoms, and the World Health Organization (WHO, 1982; Edwards et al., 1982) coined the term *neuroadaptation* to describe the permanent changes induced in the brain of an animal subjected to chronic treatments with a centrally active drug, which make the animal dependent on that drug. Individuals will depend on a drug if their psychoactive-drug consumption is associated with a behavioral pattern in which drug intake is given a sharply higher prioriy over other conducts that previously had a higher meaning for them (WHO, 1982; Edwards et al., 1982). Drug self-administration, in fact, rewards the user to such an extent that other forms of reward become meaningless. From a practical point of view, if the behavior and the health status of drug abusers become so deranged that it perturbs both their well-being and their interpersonal relationships, and if such a state is associated with criminal actions, the abused drug will be perceived by society as a real danger and consequently will merit the attention of the government, the scientific community, and the research-funding agencies.

Although people craving tobacco do not commit major crimes, pharmacokinetic data show that the rapid removal of nicotine from specific brain areas of smokers causes severe withdrawal symptoms in heavy users (Benowitz et al., 1989). Neuroadaptation to nicotine is now a recognizable neurological entity and has a true biological substratum, as discussed in the following paragraphs.

Possible Basic Mechanisms Subserving Neuroadaptation to Nicotine

Since each psychoactive drug may act on one or several synaptic processes in a single synapse, and simultaneously on several synapses engaged in different behavioral patterns, it is understandable that delineating a single common mechanism for neuroadaptation to drugs is delusory. Nevertheless, research efforts are providing some fruitful generalizations that may help to shed light on basic steps. The mesocorticolimbic dopaminergic pathway is now being recognized as a major substratum for developing dependence on psychoactive drugs. Moreover, the possible role of drug-receptor desensitization as a fundamental molecular mechanism underlying tolerance is emerging as another common denominator in neuroadaptation. A discussion on the properties of the sites to which nicotine binds within the brain is also included in this section.

The Dopaminergic Mesocorticolimbic System May Be a Convergent Pathway for Commonly Abused Drugs

People abuse drugs because they seek the pleasurable sensations that they bring about. These substances confer an "interoceptive pleasurable effect" (Di Chiara and Imperato, 1988), and animals other than humans even learn how to administer drugs to themselves (Pert and Clarke, 1987). Smokers, for example, associate their habit with alertness, easing of tensions, and muscular relaxation (Johnston, 1942).

The neurotransmitter dopamine has been asso-ciated with rewarding mechanisms of drugs (Iversen and Iversen, 1975), and experimental evidence shows that administration of commonly abused drugs provokes increased neurotransmission within specific dopaminergic pathways (Di Chiara and Imperato, 1988). The most important of these pathways is the mesocorticolimbic system, which connects substantia nigra pars compacta and other ventral tegmental neurons with the frontal cortex and the limbic system (Fallon, 1988; *see* Fig. 2).

Specifically, nicotine increases dopaminergic neurotransmission in the nucleus accumbens, a mesolimbic structure, as demostrated in vitro using brain slices (Rowell et al., 1987), or in vivo by microdialysis techniques (Mifsud et al., 1989). The latter technique was employed to show that nicotine, the opiates, cocaine, ethanol, and amphetamines increase dopaminergic transmission in the n. accumbens and the dorsal caudate. In contrast, drugs not abused by humans (atropine, antihistaminics, or antidepressants) lack this effect (Di Chiara and Imperato, 1988).

It is apparent that a better characterization of the molecular mechanisms involved in psychoactive drug effects on the dopaminergic mesocorticolimbic system may prove useful in delineating the neuroanatomical pathways involved in initiating, sustaining, and reinforcing interoceptive pleasurable sensations (*see* the review by Wise,1987).

Receptor Desensitization May Be a Fundamental Molecular Mechanism Underlying Tolerance

Specific and Nonspecific Desensitization

The fade of the biological response to applied drugs has been termed *desensitization* (*see* Ochoa et al., 1989). Since most drugs cause their effects via the operation of specific cell-membrane receptors, it is concluded that desensitization must involve some modification in the functioning of these receptors. There are responses to drugs that show desensitization (such as the release of neurotransmitter from nerve endings), but it is still not clear whether the operation of a specific receptor is implicated.

In the desensitized state, the receptor is able to bind the drug but the molecule cannot activate the receptor mechanisms involved in the production of the biological response. Desensitization to drugs can be revealed by appropriate experi-

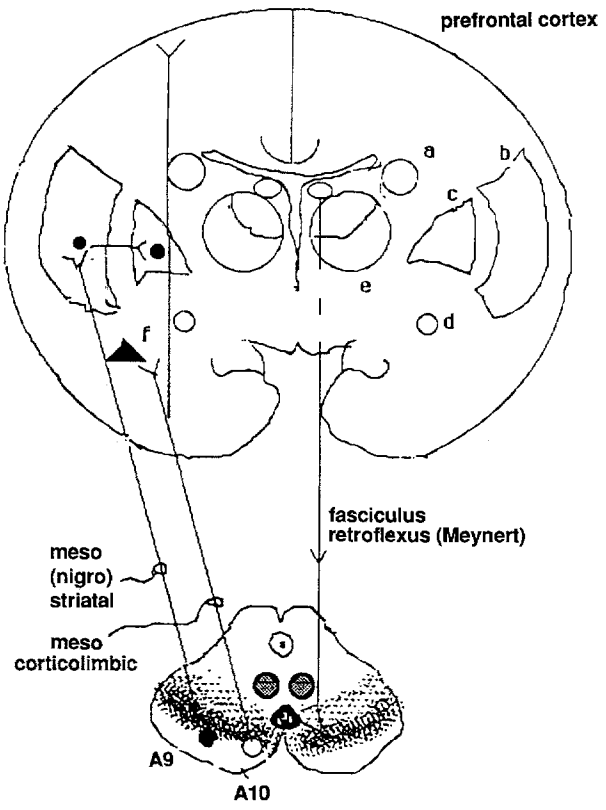

Fig. 2. Dopaminergic pathways implied in drug dependence: Two dopaminergic pathways originate in the ventral aspect of the mesenscephalon and give input to forebrain structures (mesonigrostriatal and mesocorticolimbic pathways). The mesonigrostriatal pathway originates in a group of neurons in the substantia nigra pars compacta (A9) and ends in the neostriatum, particularly the putamen. The mesocorticolimbic tract originates in a more ventral and medial area of the tegmentum (ventral tegmental area, neuronal group A10) and ends in structures pertaining to the limbic system (e.g., nucleus accumbens) and in the medial prefrontal cortex (*see* Fallon, 1988). This division is made for didactical purposes, because in fact the mesocorticolimbic tract projects to striatal structures and vice versa. An important pathway back to the mesencephalon is represented by the fasciculus retroflexus of Meynert, which originates in the habenular nuclei of the thalamus and ends in the interpeduncular nucleus of the mesencephalon. **a**, caudate; **b**, putamen; **c**, pallidus; **d**, n. basalis; **e**, thalamus (including habenula); **f**, n. accumbens.

ments, as shown in Fig. 3. The concentration of drug can be high enough to produce desensitization, even if the drug is not present during the whole stimulation period, as in A. Fade of response can also be obtained by applying several pulses of a defined drug concentration (B). Finally, desensitization can be obtained by applying a certain concentration of drug, washing it away, and, after a certain period of time, applying the same concentration (C).

When a class of drugs provokes desensitization by acting on the same, specific receptor, we speak of *specific* or *homologous* desensitization. Furthermore, when the cell or tissue becomes desensitized by different classes of drugs, each acting on separate receptors, we use the terms *nonspecific* or *heterologous* desensitization. Nonspecific desensitization is related to the cross-tolerance phenomenon described above. In this review, the emphasis will be on specific desensitization, which in turn may be subdivided into *acute* and *chronic* categories. Both types of categories have been invoked to explain the effects of nicotine on the smoker's brain.

Specific acute desensitization is a reversible phenomenon, and has rapid rates of onset and recovery. It is also known as tachyphylaxis, and the fade of the acetylcholine response after a single challenge with a high concentration of the drug is a good example (*see also* Fig. 3A). This effect is an intrinsic property of the receptor and involves a direct and specific interaction between the receptor and the ligand. The number of receptors for that particular drug remains unchanged.

Specific chronic desensitization occurs after prolonged exposure to a drug. The word *prolonged* implies more than a single drug challenge and repetitive exposure, which, in the case of human beings, often means chronic exposure. This type of desensitization is less reversible than the acute type, has slower rates of development and recovery, and, in contrast to acute desensitization, involves changs in the number of cell-surface, drug-specific receptors.

An important feature of chronic desensitization is the possible involvement of metabolic

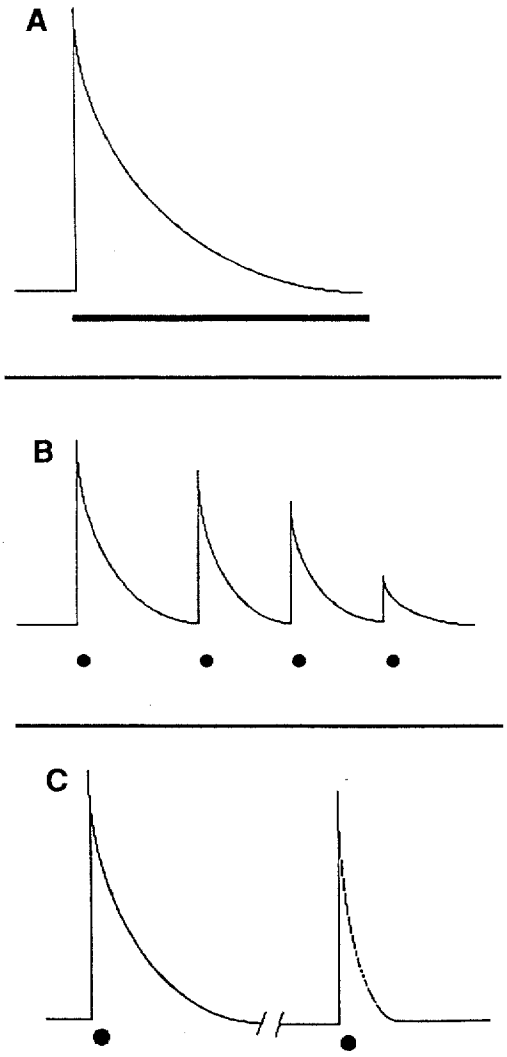

Fig. 3. Different ways to produce desensitization to drugs. Panels A–C depict a biological response obtained from a cell or a whole tissue (e.g., cell-membrane depolarization, tissue contraction, and so forth) that rises from the base line with a certain amplitude and fades as a function of time. In A the drug constantly stimulates the biological system, since it is not removed from the system (denoted by the solid bar). Note that the response decreases in a first-order fashion. In B, the same concentration of drug (dots, here denoting application and subsequent removal of the drug) is repeatedly applied to the system and the amplitude of each response also decreases as a function of time. Finally, in C the drug is applied (dot), a certain time elapses (denoted by the interrupted baseline), and the same concentration of drug is administered again (dot). In this case, the amplitude is diminished, but most imporant, the rate at which fade occurs is faster.

mechanisms triggered by the agonist and superimposed on the initial direct effects. Fig. 4 illustrates these two types of desensitization, and further examples are given later regarding nicotine. The direct and indirect effects of nicotine operate from the molecular level (e.g., desensitization) to higher levels of organization (e.g., the mesocorticolimbic pathway), bringing about long-lasting changes in brain circuitry, modifying the user's behavior, and reinforcing the idea that neuroadaptation to the drug really does take place.

Receptors For Nicotine Within the CNS

Localization

Nicotine is the classical pharmacological agent that distinguishes nicotinic acetylcholine receptors from the other major class of acetylcholine receptors (i.e., muscarinic receptors [Kerlavage et al., 1987]). These two types are found in a variety of peripheral and central tissues at cholinergic synapses, and use acetylcholine (ACh) as their endogenous ligand. The major difference between them has to do with their mode of operation: nicotinic receptors are typically fast-response cation channels, whereas muscarinic receptors are coupled to G proteins and give slower responses, usually mediated by second messengers (Strange, 1988). Both major classes of receptors have numerous subtypes defined both in pharmacological and genetic terms. Table 1 shows the localization of nicotinic receptors within the nervous system.

The neuronal nAChRs discussed so far are assumed to be located postsynaptically and depolarize the membrane through the activation of a receptor-associated ion channel, much like the fashion in which muscle nicotinic receptors are activated by acetylcholine (but *see* Wong and Gallagher, 1990). Yet there is evidence of a presynaptic localization of some neuronal nAChRs within the CNS (De Sarno and Giacobini, 1989; Rapier et al., 1990; review in Starke et al., 1989).

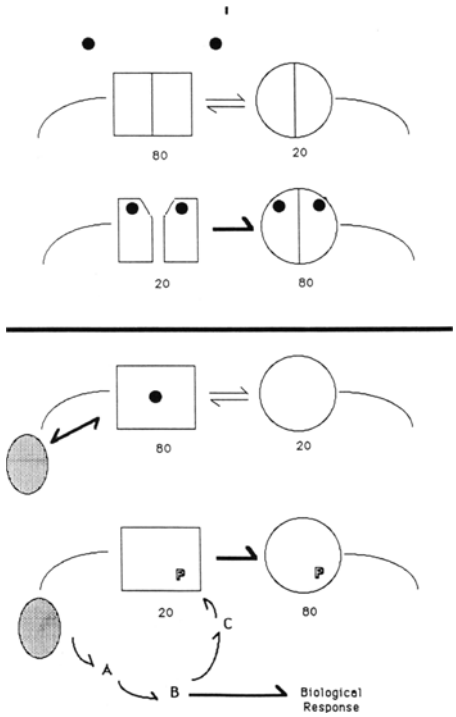

Fig. 4. Direct and indirect mechanisms for receptor desensitization. Upper panel: A membrane receptor is pictured as existing in an equilibrium of two possible conformations: the "square" (active) and the "round" (inactive) forms with a closed, built-in, ion channel. The numbers below provide an estimate of the proportion of both forms. A drug (dot, two molecules in this special case) binds to the inactive form, promotes a biological response via a conformational change that opens the channel, and displaces the equilibrium toward the inactive form. This is a mechanism by which the drug directly promotes desensitization, acting in the millisecond to second time range. The total number of receptors remains unchanged. Lower panel: Desensitization can be promoted by an indirect mechanism. The drug first binds to a receptor that is coupled to a special membrane protein (two-way arrow). This protein, in turn, initiates a series of metabolic steps (A, B, and C) that amplify the initial signal and give the biological response. The emphasis of this figure is on another metabolical event that culminates in receptor desensitization: the metabolic chain responsible for the effect also produces a covalent modification in the receptor (in this case a phosphorylation, P) provoking a conformational change leading to desensitization. Since these metabolic events occur in a different time frame (second to minute) than the events occurring in the upper panel, the drug may have dissociated from its receptor by the time this is fully desensitized. In this type of desensitization the total number of receptors may change (not shown).

The detailed subunit structures and molecular distinctions among pre- and postsynaptic receptors are still unknown. For authoritative reviews on general aspects of neuronal nAChRs the reader is referred to Lindstrom et al., 1987; Wonnacott, 1987; Connolly, 1989; Luetje et al., 1990; and Deneris et al., 1989a, 1991. We shall not discuss in detail the nicotinic receptors associated with noradrenergic nerve terminals in the peripheral autonomic nervous system (Rand, 1989) or those associated with sensory nerve terminals (Hajos and Engberg, 1988; Rand, 1989).

Binding Studies

[^{3}H]Nicotine binding sites are detected in the rat and human fetal brain as early as 16–20 d (Hagino and Lee, 1985; Yamada et al., 1986) and 12 wk of gestation (Cairns and Wonnacott, 1988), respectively. These sites have the same affinity for nicotine and lack of sensitivity to α-bungarotoxin as those found in adult animals (*see below*), mature before the establishment of synapses, and increase in density during postnatal development (Yamada et al.,1986).

In the adult brain, nicotine binds to specific CNS high-affinity binding sites (Romano and Goldstein, 1980; Marks and Collins, 1982; Marks et al., 1983; Wonnacott, 1990; Luetje et al., 1990), which coincide with the sites to which the natural neurotransmitter ACh binds (Kellar et al., 1989). One of these sites is highly stereospecific for the (–) enantiomer of nicotine and has high affinity (K_d: 1–10 n*M*). The other site displays less stereospecificity and has low affinity for the drug (K_d: 0.1–1 m*M*). Radioligand binding and molecular biology studies have confirmed that the high-affinity binding sites for nicotine qualify as functional nAChRs (Martino-Barrows and Kellar, 1987; Kellar et al., 1987; Wada et al., 1989b). Nicotine also contain high-affinity binding sites for the drug.

Pharmacological Characterization

The snake toxin α-bungarotoxin (α-BuTx), the prototypic electric-fish and vertebrate striated-muscle nAChR marker (Changeux, 1990; Pradier

Table 1
Localization of Nicotinic Receptors

Subdivision of the nervous system	Subcellular localization	Regional localization
	Presynaptic	Dopaminergic nerve endings in neostriatum and limbic system (heteroreceptors)
		Cholinergic nerve endings in hippocampus (autoreceptors)
Central nervous system	Postsynaptic	Cortical cells, nucleus basalis (Meynert), S. nigra pars compacta, and Renshaw cells (spinal cord)
Peripheral nervous system	Presynaptic	Nerve endings from motoneurones to skeletal muscle (autoreceptors)
	Postsynaptic	Skeletal muscle (motor end plate,
	Sensory nerve terminals	electroplaques of electric fish)
Autonomic nervous system	Postsynaptic	Central neurons (?)
		Postganglionic neurons of the sympathetic and parasympathetic divisions
		Chromaffin cells in adrenal medulla
	Presynaptic	Noradrenergic nerve terminals

and McNamee, 1991), is recognized as a very powerful tool for labeling and purifying the receptor. Since α-BuTx binding sites have been detected within the CNS, the natural tendency was to believe that this toxin was also suited for characterizing neuronal nAChRs, and that a detailed mapping of these receptors could be achieved by radioautographic methods that use ^{125}I-α-BuTx as the ligand. However, the toxin is unable to block the central effects of acetylcholine or nicotine (except in certain optic areas in lower vertebrates; Oswald and Freeman, 1981). Moreover, the distribution of both high-affinity [^{3}H]-nicotine and ^{3}H-ACh sites differs markedly from the distribution of ^{125}I-α-BuTx binding sites (Schwartz et al., 1982; Clarke et al., 1985; Whiting and Lindstrom, 1987).

The clear distinction between BuTx binding and high-affinity ligand binding in the CNS (Clarke et al., 1985), and antigenic divergence (Whiting et al., 1987) are key differences between neuronal and peripheral nAChRs. The physiological significance of the CNS α-BuTx binding site is still a matter of speculation (Quik and Geertsen, 1988). Recent molecular biology data suggest that the α-BuTx binding sites may be a form of nicotinic receptor, the function of which has not been yet defined (Schoepfer et al., 1990).

A special snake toxin coexists in vivo with α-BuTx, and functionally blocks nicotinic responses. This special "neuronal" toxin has been termed *kappa-bungarotoxin* (κ-BuTx [Loring et al., 1984], known also as bungarotoxin 3.1 [Chiappinelli, 1983]) and neuronal bungarotoxin. Hopefully, the term *neuronal bungarotoxin* will be abandoned, since it gives the misleading idea that neurons actually synthesize this protein. Subtle structural differences between α- and κ-bungarotoxin probably account for the different specificities toward peripheral and central nicotinic receptors (Loring and Zigmond, 1988; Chiappinelli and Wolf, 1989) (*see* Table 2). The function of neuronal nAChRs is also blocked by the competitive antagonist dihydro-β-erythroidine and the classical ganglionic blocking agents hexamethonium and mecamylamine (*see* Fig. 1; Martin et al., 1989).

Molecular Biology of Neuronal nAChRs

Neuronal nAChRs are not as abundant as peripherally occurring nAChrs, and consequently their purification is difficult. The problem has been partially circumvented by the use of molecular biology techniques, such as cloning of specific receptor subunits and their injection into *Xenopus laevis* oocytes coupled to electrophysiological recordings. This field is revealing a highly complex organization of neuronal nAChRs (Wada et al., 1988; Nef et al., 1988; Deneris et al., 1989a,b, 1991).

Skeletal-muscle and electric-fish nAChRs consist of four subunits (α, β, γ, and δ) in stoichiometry $\alpha_2\beta\gamma\delta$ (Changeux, 1990; Pradier and McNamee, 1991). Neuronal nAChRs consist of at least two subunits, termed α and β. Recent evidence suggests that the neuronal receptor could also be a pentamer (Cooper et al., 1991; *see below*). These receptors belong to a nAChR subfamily, which, in turn, is part of a gene superfamily of ligand-gated ion channels, including the glycine (Grenningloh et al., 1987) and the γ-aminobutyric acid A receptors ($GABA_AR$) (Schofield et al., 1987). All the neuronal nAChR subunit sequences that contain contiguous cysteine residues at positions homologous to 192 and 193 in the *Torpedo* sequence are classified as α subunits. Screening of the chicken genomic and rat PC12 cell (a clonal cell line derived from tumoral rat adrenal chromaffin cells, i.e., a pheochromocytoma) cDNA library using a radio- labeled probe made from a mouse-muscle α cDNA gave chicken α2 (Nef et al., 1988) and rat α3 (Boulter et al., 1986) subunit cDNAs (α1 being the mouse-muscle *a* subunit*). Further screening of chicken and rat brain cDNA libraries with the probes made from the above two neuronal subunits gave new subunits: rat α2 (Wada et al., 1988), α4 (Goldman et al., 1987), α5 (Boulter et al., 1990), and α6 (E. Lamar and J. Patrick, personal communication); chick α3, α4 (Nef et al., 1988), α5, α6 (Couturier et al., 1990a), and α7 (Couturier et al., 1990b). There is some evidence that the α5 subunit is a strong candidate for binding ^{125}I-α-BuTx (McLane et al., 1990).

Other types of subunits lack adjacent cysteines in the putative extracellular domain, correspond to the non-α *Torpedo* and muscle subunits, and are called β or non-α subunits: rat β2 (Deneris et al., 1988), β3 (Deneris et al., 1989b), β4 (Duvoisin et al., 1989), and β5, plus chick nα1 (Nef et al., 1988), nα2 (Isenberg and Meyer, 1989), and nα3 (Couturier et al., 1990a).

The rat or chick α2, α3, and α4 subunits can each be coexpressed with one of the rat β2 and β4 subunits or chick nα1 (β2) and nα3 (β4) subunits in *Xenopus laevis* oocytes yielding functional responses to either ACh or nicotine (Boulter et al., 1987; Wada et al., 1988; Ballivet et al., 1988; Duvoisin et al., 1989; Bertrand et al., 1990; Couturier et al., 1990a). More recently, human α2, α3, α4, α5, β2, and β4 subunits have also been cloned (Cimino et al., 1990; Fornasari et al., 1990; Anand and Lindstrom, 1990; Mihovilovic and Roses, 1991). Table 2 illustrates the sensitivity of the different expressed combinations of subunits to both α- and κ-bungarotoxin. Some combinations (e.g., α3β4) are not blocked by κ-bungarotoxin, the toxin presumably specific for neuronal receptors. The high-affinity binding of nicotine correlates with the receptor subtype that has the α4 agonist-binding subunit (Steinbach and Ifune, 1989), the most abundant form of neuronal nAChR in the brain. In addition, chick α7 subunit alone has been shown to be assembled into functional homooligomeric AChR receptor channels that can be blocked by α-BuTx (Couturier et al., 1990b). *In situ* hybridization studies have revealed the localization of these different neuronal nAchR subunits (for

*A cautionary note on numbering subunits: numbers immediately after a Greek letter denote a particular type of subunit making up the neuronal nAChRs. For example, α2β4 denotes a receptor having α and β subunits of types 2 and 4. The number as a subscript denotes how many subunits of that particular type are contained in a receptor molecule (e.g., a receptor consisting of two α2 and three β4 subunits would be written as: $\alpha 2_2\beta 4_3$.

Table 2
Sensitivity of Acetylcholine-Induced Depolarization to α-Bungarotoxin and κ-Bungarotoxin for Several αβ-Subunit Combinations Expressed in Oocytes [a]

	α-bungarotoxin	κ-bungarotoxin
α2β2[b]	No	No
α3β2[b]	No	Yes
α4β2[b,c]	No	Yes (but less than α3β2)
α2β4[d]	ND[e]	ND
α3β4[d]	No	No
α4β4[d]	ND	ND
α7	Yes	ND

[a] Apart from the single α7 subunit, only the α4 subunit gives a nicotinic (although feeble) response. The β2 or the β4 subunits or both β subunits expressed together also do not give a response (Duvoisin et al., 1989).
[b] Boulter et al., 1987; Wada et al., 1988.
[c] Unpublished data quoted in Duvoisin et al., 1989.
[d] Duvoisin et al., 1989. The β3 subunit (Deneris et al., 1989b) has not yet been reported tested in the oocyte expression system.
[e] ND, not determined or not yet reported.

review, *see* Deneris et al., 1991). Indirect studies (site-directed mutagenesis combined with electrophysiological studies in the oocyte expression system) indicate that the chick neuronal nicotinic receptor could consist of two α and three non-α subunits (Cooper et al., 1991).

The molecular biology studies described so far do not give any indication of the location (i.e., pre- or postsynaptic) of these different neuronal nAChR subtypes. However, *in situ* hybridization data reveal interesting differences among several areas in the brain (e.g., α4β2 is predominant in the hippoccampus and in the substantia nigra pars compacta, whereas α3β2 coexists with the former in the ventral tegmental area [Wada et al., 1989b; Deneris et al., 1991]).

Pharmacological Effects of Nicotine Involving Neuronal nAChRs

As is the case with many other abused drugs, nicotine acts at a very vulnerable target within the brain: the synapse. A diagram of a generic synapse (Fig. 5) illustrates how complicated this action can be at both sides of the synaptic cleft. Given the fact that the molecular events underlying exocytosis of transmitter-filled synaptic vesicles constitute a very complicated process (Marley, 1988; Zimmermann, 1990), it is conceivable that nicotine may influence the overall release process by modifying one or more of its constitutive steps. These include the level of free axoplasmic Ca^{2+}, the activation of specific protein kinases and protein phosphorylation (Guitart et al., 1990; Bahler et al., 1990), and probably the operation of presynaptic auto- and heteroreceptors, neuronal nAChRs, as well as other receptors, such as presynaptic GABA receptors (Ramirez et al., 1989; Kamatchi and Ticku, 1990). Moreover, postsynaptic effects of nicotine may include the triggering of the expression of specific genes (Laufer and Changeux, 1989; Morgan and Curran, 1989, Sheng et al., 1990; Sheng and Greenberg, 1990) after binding the drug to its specific postsynaptic receptors.

Presynaptic Effects of Nicotine

Nicotine acts on catecholaminergic, as well as on cholinergic, terminals modifying transmitter release. The mechanism of action is still unsolved and may involve more than one single step.

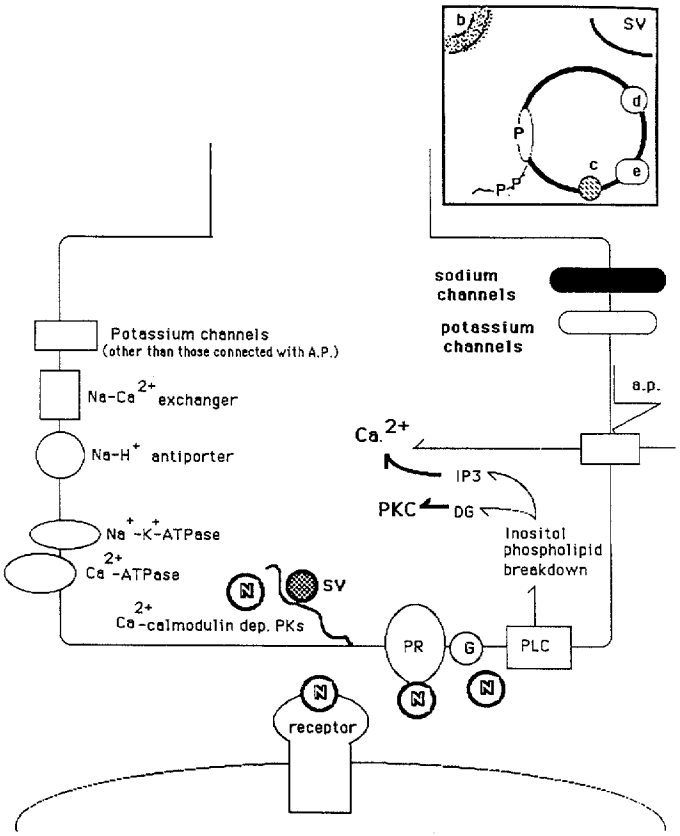

Fig. 5. A generic SNC synapse and possible sites of action of nicotine (N). There is an emphasis on membrane proteins involved in the mechanism of transmitter release, and the only subcellular organelle included is the synaptic vesicle (*see* inset). The mechanisms of synthesis or reuptake of transmitter are not indicated.

Potassium channels are entities distinct from those channels responsible for nerve impulse propagation along the axon (indicated to the right, along with sodium channels). Apart from the normal K^+- permeability, there are neurotransmitter and second-messenger-regulated channels, voltage-operated channels, a Ca^{2+}-activated channel (divided into two categories, inactivating and noninactivating), and the A channel, related to channels described in BK cells (*see also* Bartschat and Blaustein, 1985). The Na^+–K^+-ATPase is needed to sustain a normal membrane potential, and the Na^+–H^+ antiporter keeps an appropriate H^+ ion gradient between the axoplasmic and the extracellular spaces. There is also another proton-permeability of the synaptosomal membrane, which is independent of the Na^+–H^+ antiporter (Schmalzing, 1989).

Nerve endings maintain a 10,000-fold Ca^{2+} concentration gradient between the axoplasm and the extracellular fluid (Blaustein, 1988; Smith and Augustine, 1988). The Na+–C^{2+} exchanger and the Ca^{2+}-ATPase are two main proteins involved in the regulation of intraaxoplasmic free-Ca^{2+} levels (circa 0.1 μM; *see* Donnet et al., 1990). The invasion of the presynaptic nerve membrane by an action potential (a.p.) effects the opening of voltage-gated Ca^{2+} channels (right side of the figure), allowing an influx of Ca^{2+} into the axoplasm down the divalent-cation electrochemical potential gradient. The axoplasmic free-Ca^{2+} concentration suddenly increases, initiating a series of events that culminate in the liberation of neurotransmitter. The interplay between these events is still unknown, and include the operation of several presynaptic receptors (PR) and specific protein kinases (Guitart et al., 1990; Bahler et al., 1990). Two examples are provided here: presynaptic receptors might be coupled to G proteins, leading to phospholipase C activation (PLC) that induces inositol phospholipid breakdown. The latter initiates a so-called cascade of events through which inositol-1,4,5 tris phosphate increases Ca^{2+} mobilization and diacylglycerol stimulates phosphokinase C (PKC), which in turn leads to protein phosphorylation. An important phosphorylationis that effected on specific sites of synapsins through a Ca^{2+} calmodulin dependent protein kinase (Bahler et al., 1990). Inset: Once the synapsin bound to the synaptic-vesicle membrane is phosphorylated at specific sites, it detaches the synaptic vesicle from F-actin (b) or other synaptic vesicle(s) (SV). The synaptic vesicle also contains synaptophysin (Cowan et al., 1990) (c); a protein known to form transmembrane channels, the so-called mediatophore that comprises a proteolipid fraction (Israel et al., 1987) (d); and GTP-binding proteins (Zimmermann, 1990) (e). Nicotine may alter the level of synapsin phosporylation and thereby influence transmitter release *(see text).*

Effects of Catecholaminergic Terminals

Nicotine releases endogenous catecholamines from nerve terminals (Lapchak et al., 1989), chromaffin cells (Marley, 1988), and rat-tissue cultures enriched in neurons (Barochovsky and Bradford, 1987), possibly via the operation of pharmacologically defined presynaptic receptors, and with strict dependence on the presence of extracellular Ca^{2+} (Rowell et al., 1987; Rapier et al., 1988; Wonnacott et al., 1989). The effect is amply documented now in synaptosomal preparations and in vitro brain slices, and by in vivo microdi-alysis techniques.

Nicotine and other cholinergic agonists induce release of dopamine from synaptosomes and brain slices obtained from areas known to be targets for nicotine addiction. This has been verified at nigral neuron nerve terminals in the striatum (Chesselet, 1984; Pert and Clarke, 1987; Rapier et al., 1988, 1990; Wonnacott et al., 1989), an effect blocked by κ-βuTx (Schulz and Zigmond, 1989). Nicotine also enhances dopamine release from the n. accumbens (Rowell et al., 1987), and several other structures pertaining to the mesocorticolimbic system (Imperato et al., 1986), a phenomenon blocked by mecamylamine (Mifsud et al., 1989).

The putative presynaptic receptor on which nicotine would act to produce its Ca^{2+}-dependent catecholamine release is termed a *heteroreceptor*, indicating the presence of a receptive substance for nicotine in a noncholinergic terminal. The physiological significance of these heteroreceptors has been questioned, since there is not an obvious close source of ACh in areas served by dopaminergic terminals under normal in vivo conditions (Rand, 1989; Starke et al., 1989).

The noncompetitive *Torpedo* nAChR-blocker histrionicotoxin blocks dopamine release from striatal synaptosomes (Rapier et al., 1987) and catecholamine release from chromaffin cells (Wada et al., 1989a). The latter observation suggests that an ion channel might be associated with this particular type of presynaptic receptor (Wonnacott et al., 1989). Interestingly, the toxin does not block the nicotine-induced ACh release from hippocampal synaptosomes (Rapier et al., 1987; *see below*).

Effects on Cholinergic Terminals

Nicotine stimulates ACh release from cholinergic terminals obtained from postganglionic autonomic neurons (Briggs and Cooper, 1982) and cortical nerve endings (Rowell and Winkler, 1988). Presynaptic nicotinic receptors at mammalian skeletal-muscle end plates have been described as having a pharmacological profile that is closer to the brain neuronal nAChrs than to their post-synaptic counterparts (Bowman et al., 1988, 1990; Starke et al., 1989; Wessler, 1989).

Nicotine (1–10 μ*M*) enhances the release of [^{3}H]ACh evoked by nerve stimulation in a special type of rat phrenic nerve–muscle preparation (Wessler, 1989). Curare and hexamethonium block the effect, the latter antagonist being about 250 times more potent for blockade at the pre-synaptic side than at postsynaptic side (Wessler et al., 1986; Wessler and Kilbinger, 1987). It seems clear that "a full understanding of the process of transmission at the neuromuscular junction must take into account the presence of prejunctional nicotinic receptors on the motoneuron nerve terminals as well as the more familiar postjunctional receptors" (Rand, 1989).

In contrast to the presynaptic neuronal nAChRs at catecholaminergic terminals, the nicotinic receptors at cholinergic nerve endings are classified as *autoreceptors* (i.e., receptors that bind the same transmitter whose release they also control) and have been divided into two pharmacological subtypes (Bowman et al., 1990): those that cause an increased release of ACh from the nerve endings (positive feedback), and those that mediate a decrease in ACh release (negative feedback). The first type can be categorized as a good candidate for a true physiological role (e.g., ensuring a constant amount of released ACh during repetitive firing of the nerve). The autoreceptors mediating negative feedback might function in forced pharmacological situ-ations and serve little or no physiological function (Bowman et al., 1990).

Apart from nicotinic receptors, presynaptic cholinergic terminals also have acetylcholine receptors of the muscarinic type, implied in the feedback inhibition of transmitter release. Presynaptic receptors, as well as other aspects of presynaptic physiology linked to transmitter release, have been extensively documented in *Torpedo* electromotor synapses (Kloog et al. 1978; Pickard and Strange,1978; Ramirez et al., 1989; Guitart et al., 1990; Donnet et al., 1990).

An autoreceptor that modulates the release of acetylcholine has also been described in the mammalian hippocampus based on pharmacological criteria (De Sarno and Giacobini, 1989). Furthermore, hippocampal synaptosomes can be stimulated by nicotine, an effect that is not blocked by α-BuTx (Wonnacott et al., 1989).

The agonist methylcarbamylcholine (Fig. 1) appears to be quite specific for presynaptic autoreceptors (Abood and Grassi, 1986; Boksa and Quirion, 1987; Araujo et al., 1989; *see* Fig. 1). This agonist apparently discriminates between autoreceptors located in hippocampal and cortical nerve endings, but does not discriminate them from receptors located at striatal terminals (Araujo et al., 1988).

The physiology of presynaptic neuronal nAChRs may still be obscure, but the experimental evidence linking an increase in nicotine-induced dopaminergic transmission in striatal and limbic zones with interoceptive reward and increased locomotor activity is solid (*see* Pert and Clark, 1987; Rapier et al., 1987, 1988, 1990; Wonnacott et al., 1989). A better biochemical and electrophysiological characterization of these receptors is mandatory in order to determine effects falling within the time-scale of signaling events (e.g., *see* Pearce and Adamec, 1990).

Postsynaptic Effects

Traditionally, the postsynaptic effects of nicotine are much better characterized than the presynaptic effects. A postsynaptic localization of nAChRs in mesencephalic catecholaminergic neurons was suggested by experiments in which the firing rate of substantia nigra pars compacta (Clarke et al., 1987; Westfall et al., 1989) and locus ceruleus (Egan and North, 1986) neurons was increased by nicotine.

Postsynaptically located neuronal nAChRs are also enriched in cholinergic neurons projecting to areas of cerebral cortex associated with cognitive processess (e.g., intrastriatal [intracaudate] and nucleus of Meynert [basalis] neurons; Armstrong et al., 1983). About 60% of the ACh content of cerebral cortex is associated with cholinergic neurons arising from the basal forebrain (Johnston et al., 1979, 1981), and, interestingly, these neuronal groups and their related nAchRs are selectively destroyed in presenile (Alzheimer's) dementia (Coyle et al., 1983; Whitehouse et al., 1986; Whitehouse and Kellar, 1987). Acute administration of nicotine (including tobacco smoking) enhances attention and memory in humans (Wesnes and Warburton, 1983; Wesnes, 1987).

A novel postsynaptic nAChR has been described in acutely dissociated neurons from the thalamic medial habenular nucleus using patch-clamp methodology. This receptor was activated by nicotine and other agonists, and blocked by curare, hexamethonium, and mecamylamine, but was insensitive to α- and k-bungarotoxin (Mulle and Changeux, 1990). This dual insensitivity was also demonstrated in another type of neuronal nAChR located at the lateral spiriform nucleus, a chick mesencephalic nucleus (Sorenson and Chiappinelli, 1990).

Pairwise combinations of α2, α3, and α4 with β2 subunits injected into *Xenopus laevis* oocytes and patch-clamp characterization of the response to ACh revealed two distinct open states of different conductance for each assembled channel (Papke et al., 1989). Approaches like this one, or others in which subunits of neuronal nAChRs are expressed in oocytes and electrophysiological recordings of the cholinergic agonist-evoked responses are discriminated by α- and κ-BuTx, are now revealing a pharmacology much more complex than the one classically delineated in the past using hexamethonium and decamethonium (i.e., the "C-6" [ganglionic] and "C-10" nicotinic [end-plate] receptors [Fig. 1]).

Desensitization of Cholinergic Mechanisms

The multiple mechanisms responsible for chemical transmission and reception at a cholinergic synapse are potential targets for specific effects of nicotine and other cholinergic agonists leading to inactivation of the functional response. The desensitization properties of postsynaptic nAChRs have been well documented, and possible functional roles for this fundamental property of receptor operation have been advanced (Ochoa et al., 1989; Changeux, 1990; Pradier and McNamee, 1991); (Pradier et al., 1989).

From the presynaptic side of the synaptic cleft, ACh release exhibits a fade phenomenon, but the participation of presynaptic receptors in the process is less clearly defined than at the postsynaptic zone. Nicotine and cholinergic agonists could exert their effects at several steps considered crucial for mobilization of transmitter-filled synaptic vesicles (*see* Fig. 5). It seems apparent that a complete understanding of the operation of a cholinergic synapse cannot neglect either contribution (i.e., pre- or postsynaptic) in the information transmittal and reception process, and this also applies to desensitization. Table 3 (*see* pp. 268,269) summarizes some characteristics of neuronal nAChR desensitization.

Desensitization of Presynaptic Cholinergic Agonist-Regulated Neurotransmission

Like other chemical synapses within the nervous system, ACh release occurs after an influx of Ca^{2+} into the nerve ending has been initiated through voltage-sensitive calcium channels (Smith and Augustine, 1988). When the divalent cation reaches a critical axoplasmic level, via repetitive stimulation of the nerve terminal with electrical stimuli, nicotine, and other cholinergic agonists, or some of the chemical manipulations used to depolarize nerve endings (e.g., high external K^+ or veratridine), there is a fade of transmitter release that can also be termed desensitization (Katz and Miledi, 1969; Israel et al., 1987; Adams et al., 1985).

The pharmacological literature has emphasized the role of presynaptic receptors in achieving desensitization of the cholinergic response. However, the above-mentioned complexity of the complete mechanism responsible for transmitter release suggests that other processes might be involved as well.

Phosphorylation of Specific Synaptic-Vesicle Proteins

This is an important covalent posttranslational modification known to influence nerve-cell function (Huganir and Greengard, 1987, 1990; Huganir et al., 1984). Nerveending terminals contain the metabolic machinery required to carry out phosphorylations (Browning et al., 1985) that may be involved in the exocytotic process leading to neurotransmitter release (Burke and De Lorenzo, 1982; Nestler and Greengard, 1982; Llinás et al., 1985; Guitart et al., 1988). Synaptic-vesicle membrane-bound phosphoproteins, such as synapsins I and II are likely to be involved (Ueda and Greengard, 1977; Ueda et al., 1979; Hirokawa et al., 1989; Bahler et al., 1990; Volknandt et al., 1987). Dephosphosynapsin is attached to the cytoplasmic surface of synaptic vesicles and other cytoskeletal elements. The sudden increase in intracytoplasmic Ca^{2+}, which accompanies the invasion of a nerve terminal by an action potential, activates calcium calmodulin dependent protein kinase II, which phospholates synapsin I and detaches the phosphoprotein from its anchoring sites, facilitating synaptic vesicle mobilization toward presynaptic membrane active zones (Fig. 5). This was shown by Llinás (Llinás et al., 1985; Lin et al., 1990) and by injection of synapsin I and calcium-calmodulin dependent protein kinase II into the giant synapse of the squid. The increase in transmitter release evoked by the injection was suppressed by introducing the dephospho form of the protein.

In addition to synapsins I and II, other important synaptic-vesicle proteins may be involved

in synaptic-vesicle exocytosis: The so-called mediatophore (Israel et al., 1987), and the synaptophysins, integral proteins that form conductance channels and also contain sites for phosphorylation (Cowan et al., 1990).

Possible Operation of Presynaptic Receptors

Nicotine and nicotinic agonists desensitize presynaptic nAChRs from the motor nerve at the neuromuscular junction. Prolonged exposure to low (0.3 μ*M*) nicotine desensitizes the enhancement of release of ACh evoked by 10 μ*M* nicotine. Furthermore, when the motor nerve is stimulated to obtain a tetanic contraction of the muscle, two phenomena are observed: a depression of the peak amplitude of contraction (resulting from desensitization of postsynaptic nicotinic nAChRs) and a fade of the developed tetanic tension in spite of persistent stimulation of the nerve (Bowman et al., 1990). The latter effect is supposed to be mediated by rapid desensitization of the neuronal nACh autoreceptor that mediates positive feedback of ACh release. Teleologically, this desensitization process could reduce the explosive autofacilitation brought about by the operation of the presynaptic nAChR, and lead to synchronization of release sites.

Following prolonged nerve stimulation nicotinic autofacilitation ceases, whereas muscle contraction can still be observed, indicating that presynaptic receptors are more sensitive to desensitization than their postsynaptic moieties (Bowman et al., 1988, 1990; Wessler et al., 1986, 1987, Wessler, 1989).

Desensitization of presynaptic neuronal nAChRs has also been detected in several synaptosomal preparations and in chromaffin as well as PC12 cells (*see* Table 3). Interestingly, forskolin, an activator of the enzyme adenylate cyclase, antagonizes the desensitization evoked by carbamylcholine in PC12 cells (measured as the $^{22}Na^+$ efflux through a putative cation channel). This is postulated as a direct effect of forskolin, because it inhibited the binding of the noncompetitive blocker perhydrohistrionicotoxin (Nishizawa et al., 1990). There are reports of direct, non-second-messenger-mediated effects of forskolin on postsynaptic receptor desensitization (Wagoner and Pallotta, 1988; White, 1988).

Correlation Between Metabolic Phenomena of the Nerve Terminal and Presynaptic Receptor Operation

Such mechanisms as phosphorylation and presynaptic receptor operation could be single links in a complicated chain implicated in neurotransmitter release (Guitart et al., 1990; Bahler et al., 1990; *see* Fig. 5). For example, nicotine-induced release of catecholamines from chromaffin cells is correlated with phosphorylation of synapsin II (Haycock et al., 1988), and activation of β-adrenergic receptors is also linked to phosphorylation of synapsins (Browning et al., 1990). The Ca^{2+}-dependent regulation of ACh release in Torpedo nerve terminals is associated with phosphorylation of a specific 100-kDa synaptosomal protein (Michaelson et al., 1979). Both phosphorylation and release are blocked by the muscarinic agonist oxotremorine and the effects reversed by atropine, suggesting that muscarinic presynaptic AChRs have a role. The role of other presynaptic proteins, such as that of Ca^{2+}- Mg^{2+}- ATPase in muscarinic presynaptic coupling, is still not defined (Donnet et al., 1990).

Desensitization of Postsynaptic Nonneuronal nAChRs

All the essential components for receptor function are contained within a single multisubunit protein complex in the muscle and *Torpedo* nAChR (Pradier and McNamee, 1991; Changeux, 1990). The receptor does not use second messengers, G proteins, or other cellular components for its pnmary function of signal transduction, although the activity of the receptor can be modulated by a wide range of exogenous and endogenous compounds (Ochoa et al., 1989; Pradier and McNamee, 1991). Acetylcholine itself can be considered a modulator, since the natural ligand not only activates the receptor, but also triggers the conformational changes that shift the recep-

Table 3
Some Examples of the Desensitization Characteristics of Pre- and Postsynaptic Vertebrate Neuronal nAChRs

Source of nicotinic receptor	Method	μM [Desensitization] of cholinergic ligand[a]	Effect blocked by	Mode of assessing desensitization	Reference
Mouse brain	Nicotine-induced seizures following a subseizure dose of nicotine (Nic)	2.0[b] (Nic)	–	Diminished susceptibility to seizures and increased latency for onset	de Fiebre and Collins, 1988
Mesencephalic ventral tegmental area neurons	Rat brain slices, intracellular recordings	30 (Carb) 100 (Nic)	Hexamethonium, *d*-Tubocurarine	Reduction in cholinergic agonist-evoked depolarization	Calabresi et al., 1989
Acutely dissociated medial habenular cells	Patch clamp, whole cell	10 (Nic) 10 (ACh)	Hexamethonium, Curare, *d*-β-erythroidine reversible fade of current	Cholinergic agonist dose-dependent	Mulle and Changeux, 1990
Embryonic chicken sympathetic ganglion cells	Patch clamp, whole-cell mode	20(ACh)	–	Decay of the ACh-induced current	Downing and Role, 1987
Mouse neuroblastoma N1 E-115 cells	Voltage clamp	1.1 (ACh)	tubocurarine κ-BuTx	Reduction of peak inward current	Oortgiesen and Vijverberg, 1989
Chick ciliary ganglion neurons cultured cells	Patch clamp, single-channel recordings	2–5 (ACh)	–	Decrease in the frequency of channel opening	Margiotta et al. 1987
DNA library	Injection into *X. laevis* oocytes; DNA corresponding to α3 or α4 plus non-α 1	100 (ACh)	–	Decay of ACh evoked current	Couturier et al., 1990a
PC-12 cells	Carb-induced $^{22}Na^+$ uptake	1000 (Carb) 94 (Carb)	Curare	Reduction in $^{22}Na^+$influx	Simasko et al. 1986; Robinson and McGee, 1985 Boyd and Leeman, 1987; Boyd, 1987

(continued)

Table 3 (*continued*)

Nerve terminals in rat phrenic nerve	[^{3}H] Release	10–30 (DMPP) 1–10 (Nic)	Tubocurare	Fade in nicotinic agonist-enhanced, evoked ACh release	Wessler and Kilbinger, 1987 Bowman et al., 1990
	Tubocurare block in fade of tetanic contractions of skeletal muscle produced by nerve stimulation			Fade in electrically induced tetanic contractions of skeletal muscle	
Bovine adrenal chromaffin cells	Cell cultures ^{3}H-nicotine and ^{125}I-MAb35 binding Catecholamine release Rate of ^{3}H-nicotine binding	20 (Nic)	–	Increased agonist affinity to release catecholamines Increase in the rate of ^{3}H-nicotine binding by Nic preincubation	Higgings and Berg, 1988
	Monolayer culture, release of catecholamines	5 (Nic)		Decrease in the release of catecholamines	Marley, 1988 and ref. therein
Striatal synaptosomes	Continuous dopamine release from perfused synaptosomes	1.0 (Nic)	Histrionico-toxin	Decrease in dopamine release	Rapier et al., 1988, 1990

[a]Equivalent to IC_{50} or K_d values in electrophysiological and binding experiments.
[b]mg/kg body weight.

tor into a high-affinity closed-channel conformation (i.e., desensitization). A group of chemically dissimilar substances encompassed as noncompetitive blockers of receptor function directly block the open channel and/or stabilize the high-affinity desensitized state induced by activating ligands. Apart from this direct effect, noncompetitive blockers and other endogenous modulators of receptor function could operate in an indirect fashion, by facilitating the ability of acetylcholine to induce the conformational changes associated with desensitization. Most modulators appear to act by this latter mechanism, and this is acutely relevant to the operation of CNS synapses, where second-messenger-mediated effects at specific brain sites could modify desensitization of receptors to such an extent that the actual efficacy of the intervening synapses would be altered (Changeux et al., 1984; Changeux and Heidmann, 1987).

According to this molecular model, electrical signals from one neuron to the other could produce potentiation or depression of synaptic efficacy, depending on the ratio of resting and activatable receptors to desensitized receptors. The duration of these phenomena would be

determined by the kinetics of the slow allosteric transitions, and could be extended to longer timescales via covalent modifications (Changeux and Heidmann, 1987).

The desensitization characteristics of the skeletal muscle and electroplax nAChR has been described in detail (*see* Ochoa et al., 1989, and references therein; Sumikawa and Miledi, 1989; Li et al., 1990). *Torpedo californica* nAChR agonist-induced inactivation has been characterized by changes in the rate of ^{125}I-α-BuTx binding in the presence and in the absence of carbamylcholine (Carb) (Weber and Changeux, 1974; Weiland et al., 1977; Quast et al., 1978; Walker et al., 1981a; Ochoa et al., 1990), or by rapid kinetics techniques (Walker et al., 1982). The latter approach revealed two distinct kinetic processes: a fast component with a rate constant of 2–7 s^{-1} (Sakmann et al., 1980; Walker et al., 1981b, 1982; Feltz and Trautmann, 1982), and a slow component with a rate constant of 0.1–0.01 s^{-1} (Sakmann et al.,1980; Walker et al., 1981b, 1982; Heidmann et al., 1983). The fast phase of desensitization decreases the rate of ion flux across the membrane by a factor of 250; the slower component reduces ion flux to undetectable levels (Walker et al., 1982).

Nicotine acts on end plates as a typical cholinergic agonist, although the concentrations necessary to activate the receptors are higher than those of acetylcholine. Desensitizing doses for the *Torpedo* receptor range from 5 μ*M* to 1 m*M* for Carb, but are higher for (–) nicotine. Table 4 shows a comparison between the desensitizing properties of nicotine and Carb expressed by purified *Torpedo* nAChR reconstituted into lipid vesicles.

Modulation of Postsynaptic Nonneuronal Receptor Desensitization

One of the most important properties of desensitization is its propensity to be modulated by chemically dissimilar substances, such as exogenous noncompetitive blockers of receptor function, and endogenous substances, such as calcium or special peptides. This is particularly relevant to the neuronal nAChR, since peptides coexist with neurotransmitters in the same neuron (Hokfelt et al., 1980, 1986) and even in the same synaptic vesicle (Pelletier et al., 1981). Modulation could take place through a direct effect on cholinergic-agonist-induced desensitization, or through an indirect mechanism that triggers the production of second messengers (Fig. 4). The most important consequences of these metabolic changes are phosphorylation and Ca^{2+} mobilization.

Phosphorylation and Calcium. Calcium has an important and direct role in receptor desensitization. The possible functional relevance of phosphorylation has been recently established by data that demonstrate the modulation of receptor activity by kinase stimulation (Downing and Role, 1987; but *see* Montal and Montal, 1987). These two topics will not be discussed here and the interested reader is referred to Huganir et al., 1986; Huganir and Greengard, 1987,1990; Hopfield et al., 1988; Hemmings et al., 1989; Ochoa et al., 1989; Pradier and McNamee, 1991). Phosphorylation effects are also characterized in neuronal preparations (Rubin et al., 1988; Schuetze and Role, 1987).

Apart from having direct functional effects on the ion channel, phosphorylation might also regulate nAChR assembly. Delta subunits assembled in pentameric complexes have lower phosphorylation levels than their monomeric counterparts (Ross et al., 1987). It is important to note that the α4 subunit has a large cytoplasmic segment with potential sites for phosphorylation (Ross et al., 1988). Until now, there has been no direct correlation between in vivo phosphorylation and the rate of desensitization.

Substance P. This undecapeptide (Pernow, 1983) has direct and indirect effects on neuronal nAChRs (Stallcup and Patrick, 1980; Clapham and Neher, 1984; Role, 1984; Boyd and Leeman, 1987; Weiland et al., 1987; Simasko et al., 1985, 1987; Higgins and Berg, 1988; Simmons et al., 1990). It is most likely that the predominant effects of substance P are exerted via phosphorylations of specific proteins.

Thymopoietin (Tpo). Thymopoietins I and II are structurally related polypeptides of 49 amino

Table 4
Effects of (–) Nicotine on Reconstituted nAchR Desensitization[a]

	Cholinergic agonist	
	Nicotine	Carbamylcholine
Efficacy, m*M* [b]	1.0	0.3
EC_{50}, m*M* [c]	0.2	0.03
K_{low}, m*M* [d]	20	10
K_{high}, m*M*	8	ND
DD, m*M* [e]	3.0	1.0
$T_{1/2}$, s	30	10

[a] *Torpedo californica* nAChR was purified as described in Ochoa et al. (1990) and mixed (1.3–1.5 mg/mL) with lipids and cholate to give 2% detergent and a final concentration of lipid and receptor of 20 mg/mL and 1.0 mg/mL, respectively, and dialyzed against cholate-free buffer. The nicotine- and carbamylcholine-stimulated uptake of $^{86}Rb^+$ into vesicles was used as a qualitiative indicator of functional responses (Jones et al., 1987). The relative rate of ^{125}I-α-BuTx binding in the absence and in the presence of 1–15 μ*M* Carb/nicotine, or the inactivation of the 1–2 m*M* Carb/nicotine-induced ^{86}Rb influx produced at 1–10 μ*M* Carb or nicotine was monitored as described in Walker et al., 1981b, and Ochoa et al., 1990. Abbreviations: EC_{50}: Effective concentration 50; DD: desensitization dose; and $T_{1/2}$: Empirical half time for desensitization in the toxin binding assay (Ochoa et al., 1988,1990).

[b] Calculated as the dose that induces maximal response (flux assay).

[c] Calculated as the dose that induces 50% of the response (EC_{50}: effective concentration 50) (flux assay).

[d] Toxin binding assay. For Carb the data are from Ochoa et al., 1990.

[e] Calculated as the dose that induces fade of response (DD: Desensitization dose) (flux assay).

[f] $T_{1/2}$: empirical half-time for desensitization (Ochoa et al., 1988).

acids isolated from bovine or human thymus (Audhya et al., 1981, 1987). The hormone (and its putative active center of five amino acids) blocks neuromuscular transmission in, and binds to, the sites at which α-BuTx binds in Torpedo C2 cells (*see* Ochoa et al., 1989, and references therein; Revah et al., 1987; Ochoa et al., 1988, 1990; Quik et al., 1990c, 1991a). In regard to the nervous system, Tpo has been detected in mouse spinal-cord and brain homogenates, in supernatants of a mouse neuroblastoma cell line, and in rat brain (Brown et al., 1986; Quik et al., 1991b). Tpo specifically binds to the nicotinic neuronal α-BuTx binding site (Quik et al., 1989, 1991b; Lukas et al., 1990), and to α-BuTx binding sites in chromaffin and PC12 cells in culture (Quik et al., 1990a,b).

These effects of Tpo have considerable relevance, since recent data show that there is a special neuronal nAChR α subunit responsible for α-BuTx-binding (McLane et al., 1990), and there is evidence indicating that α-BuTx binding sites in the brain may be actual members of the nAChR family (Schoepfer et al., 1990).

Calcitonin Gene-Related Peptide (CGRP). CGRP, a peptide of 37 amino acids, is the product of the posttranslational processing of a 16-kDa protein encoded by an mRNA related to the calcitonin gene, and coexists with ACh in the CNS (Changeux, 1986; Hokfelt et al., 1986). CGRP increases the level of surface nAChR in primary chick muscle cells in culture (Fontaine et al., 1986; New and Mudge, 1986), and augments the ex-

pression of an nAChR α subunit (Fontaine et al., 1987; Klarsfeld and Changeux, 1985). CGRP has been postulated to be a nerve-derived trophic factor that regulates nAChR biosynthesis (Laufer and Changeux, 1989). The peptide indirectly modulates nAChR desensitization in a mouse muscle cell line (Mulle et al., 1988).

Desensitization of Postsynaptic Neuronal nAChRs. Neuronal receptors tend to desensitize readily, whereas muscle receptors are less readily desensitized (Paton and Savini, 1968; Clarke et al., 1987). Table 3 summarizes some properties of postsynaptic neuronal nAChR desensitization, including cells in culture and expressed subunits in oocytes.

One of the key questions in research on central nicotinic receptors has to do with the possible existence of an equilibrium between desensitized (high-affinity) and nondesensitized (low-affinity) forms of nicotinic receptors, as is the case for the muscle and electric-fish (*Torpedo*) receptor (Ochoa et al., 1989; Changeux, 1990; Pradier and McNamee, 1991). Alternatively, there could be two types of receptor, each with different affinities for nicotine. Finally, it is not known whether different receptor subypes within the brain have different desensitization properties.

Nicotine addiction may involve all or only a subset of the different nicotine binding sites present in the CNS, and receptor subtypes that are most sensitive to nicotine-induced desensitization may play a key role in mediating the effects of nicotine. Sumikawa and Miledi (1989) have shown that cat-muscle and *Torpedo* nAChR desensitize at different rates, and coexpression experiments demonstrate that desensitization depends on the γ subunit of the muscle-type receptor.

Recently we found that change of extracellular pH can modulate nAChR desensitization and that the pH dependence of desensitization is different for *Torpedo* and mouse-muscle nAChRs expressed in *Xenopus* oocyte (Li and McNamee, submitted). For *Torpedo* nAChR, both decreasing and increasing pH from 7.4 increases the desensitization rate, with two apparent pK_a values of 6.5 and 9.5, whereas for mouse-muscle nAChR, the desensitization rate remains unchanged from pH 6.5 to pH 9.0 and increases at more acidic pH, with a pK_a of 4.7. This difference in the pH-dependence of the desensitization rate between the nAChRs from two species expressed in the same environment suggests that the specific amino acid residue(s) involved in the allosteric transition from open state to desensitized state is different for these two nAChRs. Further pH studies using mouse–Torpedo hybrid receptors from mixing and matching the subunits from these two species suggest that the characteristic acidic group (Glu or Asp) determining the pH-dependence of mouse-muscle nAChR desensitization is apparently associated with mouse-muscle nAChR β subunit.

Recent experiments by the group of Berg (Vijayaraghavan et al., 1990) show that in chick ciliary ganglion neurons 50- and 58-kDa components of the nicotinic receptors (derived from α3 subunits) are phosphorylated via a cAMP mech-anism. The striking difference is that in the *Torpedo* nAChR phosphorylation occurs in non-α subunits and the consequence is desensitization, whereas in this type of neuronal receptor phosphorylation is associated with enhanced responses to cholinergic agonists.

Desensitization could have different characteristics in different areas of the brain. For example, receptors possessing the chicken α4 desensitize slowly, whereas those containing the chicken α3 desensitize quickly (Couturier et al., 1990a).

A Phenomenon Unique to Neuronal nAChRs: Upregulation of Cholinergic Binding Sites

When most drug receptors are chronically overexposed to their specific agonists, their number decreases. The same effect can be achieved through suppression of the agonist-inactivation mechanisms (e.g., inhibition of specific agon-ist-

cleaving enzymes, or blockade of agonist reuptake). This agonist-induced decrease in the number of cell-membrane receptors is called downregulation (Creese and Sibley, 1981; Sibley et al., 1987; Wonnacott, 1990).

Chronic administration of specific drug-receptor antagonists results in receptor underexposure to the agonist and leads to an increase in the number of cell-membrane receptors. This can be similarly obtained by reducing the agonist availability at the synaptic cleft. This induced increase in the number of cell-membrane receptors produced by reduced availability of agonist is called upregulation.

As far as nicotine and neuronal nAChRs are concerned, downregulation is seen after chronic treatment of rats with cholinesterase inhibitors (i.e., equivalent to blocking inactivation of the agonist acetylcholine; Schwartz and Kellar, 1983, 1985). However, in violation of established pharmacological rules, chronic treatment of rodents with nicotine increases, or upregulates, the density of cholinergic ligand binding sites, whereas the affinity for cholinergic ligands remains unaltered (Schwartz and Kellar, 1983, 1985; Marks and Collins, 1982, 1985; Marks et al., 1983; Collins and Marks, 1987; Nordberg et al., 1989; Grenhoff and Svensson, 1989; Kellar et al., 1989). It is not known whether these new sites are presynaptic, postsynaptic, or both.

This fundamental observation has an important human correlate: brain samples obtained from autopsies performed on persons who were heavy smokers show an increase in cholinergic binding sites (Benwell et al., 1988). Also, brains from fetuses whose mothers were smokers also show such an increase in cholinergic binding sites (Cairns and Wonnacott, 1988).

Brain nicotinic receptors are unique in their agonist-induced upregulation, because chronic exposure to nicotine actually downregulates nicotinic receptors in peripheral tissues containing nAChRs (Robinson and McGee, 1985; Berg et al., 1989). From a behavioral point of view, the upregulation of neuronal nAChRs is associated with tolerance in rodents (Collins et al., 1988a, and references therein). Tolerance to, dependence on, and withdrawal from nicotine are also described in human subjects (Tennant et al., 1983; APA, 1987; DHHS, 1988; Benowitz et al., 1989).

Possible Mechanisms

Agonist-induced receptor desensitization has been suggested as the signal that triggers up regulation (Schwartz and Kellar, 1985; Marks and Collins, 1985; Collins and Marks, 1987) and recently desensitization of specific presynaptic neuronal nAChRs has been postulated as an important mechanism for developing tolerance to nicotine (Rapier et al., 1988; De Sarno and Giacobini, 1989). Upregulation implies increased synthesis of receptors, and it is important to mention, in this respect, that nicotine induces rapid transcription of early response genes (e.g., *c-fos*; Greenberg et al., 1986; Bartel et al., 1989; Morgan and Curran, 1989; Sheng et al., 1990; Sheng and Greenberg, 1990).

The exact mechanism coupling membrane-bound, desensitized neuronal nAChRs to an augmentation of their synthesis has not yet been resolved, but it appears that a desensitized, inactive neuronal nAChR constitutes a key signal to the nucleus that activates gene expression (*see* Laufer and Changeux, 1989; Greenberg et al., 1986; Bartel et al., 1989; Morgan and Curran, 1989; Sheng et al., 1990; Sheng and Greenberg, 1990). This increased synthesis of receptor triggered by its own inactivation would consitute the molecular basis underlying the smoker's tolerance to, dependence on, and withdrawal from nicotine. It would be expected that mecamylamine, a specific antagonist of neuronal nAChRs (Fig. 1), would improve the symptoms of smoking withdrawal, as suggested by Tennant et al. (1983), but the evidence is tenuous.

Although the desensitization explanation is favored by many to explain the upregulation phenomenon, other alternative hypotheses (which merit being put to experimental test) have been advanced. For example, nicotine might

interfere with either the synthesis or the release of ACh, producing upregulation by the well-established mechanism of agonist depletion at the synaptic cleft (Schwartz and Kellar,1983). Alternatively, a metabolite of the drug (e.g., cotinine) might act as a functional antagonist of neuronal nAChRs (Schwartz and Kellar, 1983).

Desensitization of Cholinergic Function and Neuroadaptation to Nicotine: A Hypothesis

It is proposed here (partly based on Collier, 1966) that the initial event involved in rapid transcription of early-response genes (Greenberg et al., 1986; Bartel et al., 1989; Morgan and Curran, 1989; Sheng et al., 1990; Sheng and Greenberg, 1990) is neuronal nAChR desensitization provoked by a chronic regime of nicotine administration. The number of neuronal nAChRs originally exhibiting low affinity for nicotine could increase as a compensatory response to the nicotine-induced transition of the receptors to a high-affinity, inactivatable (i.e., desensitized) form (Schwartz and Kellar, 1983; Marks et al., 1983).

That this sequence has a defined temporal sequence is reinforced by two facts (1)repeated administration of nicotine is required to observe receptor upregulation (Schwartz and Kellar, 1985), and rapid desensitization *per se* is not enough to bring about the phenomenon; (2) pre- and postsynaptic neuronal nAChRs are regulated through retrograde signals from the synaptic target tissue, via presynaptic inputs, and by the activation of second-messenger-regulated metabolic cascades (Schuetze and Role, 1987; Berg et al., 1989; Laufer and Changeux, 1989), phenomena that occur well beyond the time-frame of normal neurotransmission. It is reasonable to conceive that nicotine, apart from having a direct effect of its own on cholinergic neurotransmission, may trigger key signals that initiate long-term, second-messenger-mediated effects (Rubin et al., 1988; Changeux and Heidmann, 1987; *see* Fig. 4).

The hypothesis makes the economical assumption that the basic principles of receptor desensitization of the nicotinic receptor family are maintained among its members. Consequently, Changeux's minimal four-state model to explain electroplax-nAChR desensitization (Changeux and Heidmann, 1987; Changeux, 1990) can be applied to the neuronal nAChR, assuming that it may exist in (1) a resting, low-affinity, activatable state; (2) a cholinergic-agonist-activated state; and (3) a high-affinity, desensitized (inactive) state. For the sake of simplicity, the existence of a fourth, intermediate, desensitized state (Changeux and Heidmann, 1987; Changeux, 1990) will not be discussed in the present context.

In the initial stages of the nicotine-administration period, the drug activates predominantly low-affinity postsynaptic nAChRs producing depolarization at autonomic ganglia (Eccles, 1935), muscle (Thesleff, 1955), an increase in the cognitive processing activities of the brain via activation of brain basal structures (Wesnes and Warburton, 1983; Wesnes, 1987), and rewarding effects promoting repetitive drug administration as a result of stimulation of dopaminergic ventral tegmental neurons (Bozarth, 1986; Calabresi et al., 1989). These behavioral effects are related to the increase in alertness and mental speed experienced and referred to by stressed smokers.

By displacing the equilibrium between the possible configurations of the receptor toward a high-affinity form, nicotine acts as a functional (not pharmacological) antagonist, and provokes a rapid inactivation of receptor function (e.g., the muscle relaxation seen during smoking). Provided presynaptic neuronal nAChRs are involved in neurotransmission, their propensity to desensitization causes a diminished supply of acetylcholine to the synaptic cleft, effected by several (still unresolved) steps intervening between presynaptic receptor desensitization and decreased transmitter release.

It is proposed that one of these fundamental steps relating nicotine-induced desensitization of transmitter release could be correlated with

changes in the state of phosphorylation of specific synaptic-vesicle proteins. Nerve-ending terminals contain the metabolic machinery required to carry out phosphorylations (Browning et al., 1985) involved in the exocytotic process leading to neurotransmitter release (Burke and De Lorenzo, 1982; Nestler and Greengard, 1982; Llinás et al., 1985; Guitart et al., 1988; Volknandt et al., 1987; Hirokawa et al., 1989; Bahler et al., 1990). It has been shown that synapsin phosphorylation can be correlated with the nicotine-induced release of catecholamines from chromaffin cells (Haycock et al., 1988) and with activation of β-adrenergic receptors (Browning et al., 1990).

If there exists a close relationship between transmitter release and synapsin phosphorylation, a synaptosomal preparation desensitized by nicotine should contain synapsin molecules with low levels of phosphorylation. If this assumption is correct, transmission could be regained by injecting phosphorylated synapsin into the synaptosomes. These experiments are feasible, since frozen-thawed rat-brain synaptosomes (in the presence of 5% dimethylsulfoxide) can be loaded with the metabolic apparatus required for phosphorylation (Nichols et al., 1989).

Desensitization of presynaptic nAChR heteroreceptors at mesocorticolimbic dopaminergic terminals (Rapier et al., 1990) may be responsible for the behavioral phenomenon of nicotine dependence. The end result of these pre- and postsynaptic desensitizations is that higher doses of nicotine are required to sustain both pre- and postsynaptic effects. Tolerance to nicotine would then follow.

As an epiphenomenon, inactivation of pre- and postsynaptic nAChRs constitute a trigger for the synthesis of new receptors (i.e., upregulation) through effects operating at the transcriptional level, which also involve phosphorylation of target proteins. The phosphorylation mechanisms that are implied in stimulus–secretion coupling (Marley, 1988) are also implied in the process of stimulus–transcription coupling (Sheng et al., 1990).

The newly synthesized receptors become inactivated as soon as they reach the neuronal plasma membrane, since nicotine will displace receptor equilibria towards the inactive, desensitized form. If the administration of nicotine is suddenly curtailed, there is overall CNS excitability and symptoms opposite of those registered during the chronic-administration period (i.e., the withdrawal syndrome), probably because there is a relative increase in the supply of transmitter with respect to the number of active postsynaptic nAChRs. The latter implies that the inactivation process is reversible and that recovery of presynaptic cholinergic mechanisms recover easily compared to postsynaptic ones (Collier, 1966).

Superimposed onto the complexity of these mechanisms is the possible modulation of neuronal nAChR desensitization exerted by other agents, such as the above-discussed substance P or thymopoietin, drugs that are endogenous to the body and could be engaged in the physiological modulation of these receptors.

Chronic Administration of Nicotine and the Developing Nervous System

Although the deleterious effects of commonly abused drugs are studied mainly in adolescent and adult individuals, the mechanisms by which they impact a developing nervous system has been underappreciated and is now recognized as a potential danger. The number of pregnancies associated with drug addiction is escalating at an alarming rate, and there is an urgent need for abundant research on this subject.

Pregnant mothers exposed to nicotine may show a different pattern in the aforementioned hypothetical sequence of events, since the full range of actions of nicotine on key neuronal maturation steps (Ochoa et al., 1982; Yamada et al., 1986) or neurotransmitter systems (Slotkin et al., 1987a,b) are still unknown. In this respect, we found a novel nicotine-gated cation channel in

Xenopus laevis oocytes (Li and McNamee, manuscript in preparation). The activation of this channel requires nicotine in the millimolar range with a Hill coefficient of about 1. The nicotine-induced current response cannot be blocked by either α- or κ-BuTx.

Nicotinic acetylcholine receptors mature before the development of presynaptic cholinergic neurons (Yamada et al., 1986), and maternal smoking poses serious problems to fetal, perinatal, child, and adolescent life. Cigaret smoking during pregnancy results in intrauterine growth retardation and is correlated with attention-deficit disorders and other cognitive syndromes of postnatal life (Butler and Goldstein, 1973; Brown et al., 1985; Fung, 1988; Kristjansson et al., 1989). Alterations in cognitive mechanisms induced by nicotine are well documented in adult subjects.

Perinatal exposure to nicotine may be extremely damaging to a developing nervous system, either hemodynamically (hypoxia-ischemia) or through direct effects on nerve cells (Slotkin et al., 1987a,b; Navarro et al., 1989). True [^{3}H]nicotine binding sites appear in mammalian brain at early stages of development (Hagino and Lee, 1985; Cairns and Wonnacott, 1988). Pregnant animals and their offspring exposed to high levels of nicotine also show upregulation of nicotinic (but not muscarinic) binding sites (Hagino and Lee, 1985; Slotkin et al., 1987a). It has been suggested that the phenomenon is also triggered by desensitization of neuronal nAChRs (Cairns and Wonnacott, 1988). The question of how reversible these perturbations may be is now being posed to researchers and to physicians attending the infants referred to (unfortunately) as drug babies by the popular press.

Conclusion

Nicotine produces neuroadaptation by interacting with cholinergic mechanisms within the CNS. The intervening steps of this complicated process are unknown, but significant advances have been made in the last decade regarding the characterization of the nAChR family. The structural and pharmacological data provided by molecular genetics give an idea of how formidably complex these receptors are. The detailed subunit structures and molecular distinctions among pre- and postsynaptic neuronal nAChRs and the properties of their functional inactivation are still unknown. Molecular information about the individual components of the multiple steps leading to acetylcholine release, and their desensitization properties, is also lacking.

Research efforts in the next years will address such problems as the possible iinkage between desensitization of cholinergic function and neuroadaptation to nicotine through cell and molecular biological approaches, as well as correlative animal behavioral studies. The effects of nicotine and other commonly abused drugs on the developing nervous system will be another major area of research. As an important corollary of these endeavors, scientists and the general public will be able to understand the neurobiological basis for the interoceptive pleasurable effects of their rewarding (drug- and non-drug-related) sensations.

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